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SPECIATION AND HUMAN HEALTH RISKS EVALUATION OF NICKEL AND CADMIUM IN LAND AND MARINE SNAILS FROM BAYELSA STATE, NIGERIA.

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ABSTRACT

The toxicity of heavy metals, their environmental mobility, bioavailability and tendency to accumulate in living systems are strictly correlated to their chemical forms (species) rather than total concentration. Usually, knowledge of total concentration of heavy metals give poor information about their potential risk in the environment and biota. However, this study investigated the speciation of Nickel and Cadmium concentrations in land snails (*A. achatina* and *L. flemmea*) and marine snails (*P. aurita* and *T. fuscatus*) from Bayelsa State, Nigeria, and the human health risks association via the consumption of these snails. The concentrations of Ni and Cd in the snails in the various fractions were analyzed using FAAS (GBC Avant aver 2.02 model). The non-polar and polar fractions of Ni and Cd concentrations ranked the highest in the snails. The concentration of Ni in all the snails in each fraction were higher than the limits set by regulatory bodies, while Cd concentrations were higher only in *A. achatina* in all the fractions except the residual fraction which was below the limits. Human health risk evaluation results showed that, the exposure to Ni and Cd via these snails will pose no carcinogenic and non-carcinogenic risks at the moment, however, continuous and excessive daily consumption above 0.025 mg/kg/bw-day may pose health risks in future. Therefore, moderate intake of these snails is recommended.

KEYWORDS: Speciation, Health Risk, Nickel, Cadmium, Snails.

1.0 INTRODUCTION

Several studies have focused on human exposure to toxic heavy metals through the consumption of contaminated food.[1,2,3,4] However, most of these studies fail to consider the fact that these toxic metals exist in different chemical forms or species.

Templeton *et al.*,[5] defined "***chemical species*** as the specific form of an element such as isotopic composition, electronic or oxidation state, and/or complex or molecular structure of an element. Also, they defined "***Speciation of an element***" as the distribution of an element amongst defined chemical species in a system and "***Speciation analysis***" as the analytical activities of identifying and or measuring the qualities of one or more individual chemical species in a sample. Therefore, the need for speciation analysis arises from the necessity to determine the concentration of species characterized by the highest toxicity, mobility, bioavailability and bioaccumulation in an environmental matrix.[6] The toxicity of heavy metals, their environmental mobility and tendency to be accumulated in living systems are strictly correlated to their chemical forms rather than total concentration. Usually, knowledge of total concentration of heavy metals give poor information about their potential risk[5] in the environment and biota. This has led to the development of two major approaches; namely; "***Organometallic speciation***" where metals with different oxidation states are characterized by different toxicity, mobility etc mainly Pb, Hg, Sn and Ni (e.g tributyltin, methyl mercury, tetraethyl lead, nickel tetracarbonyl, nicklelocene etc) and "***inorganic speciation***" where, metals with different oxidation state are also characterized by different toxicity, mobility etc, mainly Cd, Cr, As, Se, Ni and Sb (e.g cadmium hydroxides, cadmium chlorides, nickel hydroxides, nickel chlorides, nickel sulfate, nickel nitrate, and other chlorides, hydroxides, ion etc).[7]

Speciation can also be based on the polarity of the species. Polar species are those that preferably dissolve in a polar solvent while non-polar species dissolve in a non-polar solvents. Generally, inorganic species are said to be polar while organic or organometallic species are said to be non-polar [8]. However, some organic species with smaller number of organic substituents exhibit some degree of ionic character and have been found soluble in polar solvents examples; monobutytin and methylmercury. The knowledge of the distribution of polar and non-polar species of metals in an organism will give us a better understanding of the toxic-kinetics of these metals when they enter the human system. Literature revealed that polar species will likely enter the blood plasma and brain fluid while the non-polar species will likely be found in the body lipids.[8] Therefore, the objectives of this study is to determine the speciation (chemical forms) of Ni and Cd using sequential chemical (water, hexane, and methanol/DCM) extraction procedures, and assess the health risk association with exposure to Ni and Cd compounds via the consumption of land snails (*A. achatina* and *L. flammea*) and marines (*P. aurita* and *T. fuscatus*). The concentrations of Nickel and Cadmium in each fraction was determined using FAAS.

2.0 MATERIALS AND METHODS

2.1 Description of Sampling Sites

Bayelsa State lies in the heaviest rainfall area in Nigeria with heavy rain forest and a short dry season (from November to March) the State is geographically located within Latitude 04^{O} 15'' North, 05^{O} 23''South and Longitude 05^{O} 22'' West arid 06^{O} 45'' East. Bayelsa State shares boundaries with Delta State in North, Rivers State in the East and the Atlantic Ocean in the West and South. The State lies in the tropical rain forest belt with and area of about 21110 square kilometers. More than three quarters of this area is covered by water with a moderately low land stretching from Ekeremor to Nembe. The major occupations in the State are fishing, farming, palm wine tapping, local gin, trading, carving & weaving. Bayelsa State is also one of the Coastal areas of Nigeria where there is massive oil exploitation and exploration, accounting over 30% of the Nigerian oil production. There are hundreds of oil well and flow station across the state. These and many more other activities have no doubt released heavy metals into the terrestrial and aquatic environments of Bayelsa State.

Fig 1: Map of Bayelsa State.

2.2 Collection and Preparation of Samples

Land snails, (*Achatina achatina* and *Limicolaria flammea* (commonly known as giant land and garden snails), and marine snails; *Pachymelenia aurita* and *Tympanotonus fuscatus* (commonly called periwinkles) were purchased from commercial sellers from Yenagoa, main market, Bayelsa State, Nigeria. These snail species are cherished among local consumers due to its high protein, minerals and vitamin contents and are readily available.[9,10] They are easily handpick, hence serve as a cheap sources of animal proteins for many coastal communities in Nigeria.[11] The snails were washed with cold water and wrapped in a polythene bags, labeled accordingly and transported to the University of Port Harcourt, and identified at the Department of Animal and Environmental Biology. Thereafter, the snail shells were cracked to obtain the whole soft tissues (edible tissues). Snail tissues were thoroughly washed several times with distilled water, over dried at 105^{0}C to a constant weight. Then the oven dried samples were ground and sieved to obtain a uniform particle size.

A. Achatina *L. Flammea* *P. aurita* *T. Fuscatus*

Fig. 2: Land snails (*Achatina and L. Flammea*), and marine snails (*P. aurita and T. Fuscatus*).

2.3 Speciation Analysis

Speciation analysis of Ni and Cd in snail samples were carried out using three stage sequential extractions produced with water, hexane and methanol/DCM(8:2) to obtain;

1. Water soluble fraction, WF_1
2. Non-polar (hexane) fraction, NF_2
3. Polar (methanol) fraction $PF_{3\ (12)}$

2.3.1 Water Soluble Fraction, (WF_1)

1g of each biomas was weighed into extraction bottle and 15ml of distilled was added. The mixtures were placed on electrical shaker for twenty four (24) hours, after which they were removed, filtered and the water soluble fractions were obtained. Then, the residues were dried at room temperature for two (2) days.

2.3.2 Non-Polar (Hexane) Fraction (NF_2)

10ml of hexane was added to each residue obtained from fraction one (F_1). All mixtures were placed on electrical shaker for twenty-four (24) hours, thereafter the mixtures were filtered and the non-polar (hexane) extracts were obtained. The residues were also dried at room temperature for two (2) days.

2.3.3 Polar (Methanol) Fraction (PF_3)

10ml of methanol/DCM (8:2) was added to the dried residues of F_2 and placed on a shaker for twenty-four (24) hours. Thereafter, the mixtures were filtered and the polar or methanol extracts were obtained. Again, all residues were dried at room temperature for two (2) days.

2.3.4 Digestion of the (Residue) Fraction (RF_4)

The dried residues were digested with 10ml of aqua regia (3:1 v/v of Conc HCl/HNO_3), and 1ml of $HCLO_4$. The digest were diluted with 25ml distilled water and filtered to obtain the residual fraction (F_4).[13]

The concentrations of Ni and Cd in each extract were analyzed by Flame Atomic Absorption Spectrophotometer, FAAS (GBC Avant aver 2.02. Quality control of metals analysis in this study was checked by reagent banks, triplicates samples, a certified reference material solutions provided by NRC, Canada, and triplicate metals determination. Recoveries of metals ranges from 86%-99% for Ni and 100%-103 for Cd, which indicates good accuracy and result precision.

2.4 Human Health Risk Evaluation

The human health risk evaluation of Nickel and Cadmium via snail's consumption were assessed using the Chronic Daily Intake (CDI), Target Hazard Quotient, (THQ) for non-carcinogens, Hazard Index (HI) for the combined hazard of Ni and Cd in the snails, and Target Carcer Risk (TR_c) for carcinogens established by USEPA, Region III Risk-based concentration table I.[14,15,16] The equations used for estimating the human health risks of Ni and Cd via snail consumption for this study are expressed as follows;

2.4.1 Chronic Daily Intake (CDI) = CDI (mg/kg/bw/day)

$$= \frac{MI_s\ X\ MC_{(s)}}{BW_a} \text{ ....i}$$

2.4.2 Target Hazard Quotient (THQ_n) THQ_n

$$= \frac{EF\ \ X\ ED\ X\ MI\ \ X\ \ MC_S}{RFD\ \ X\ \ BW_a\ \ X\ \ AT_n}\ X\ 10^{-3} \text{ ....ii}$$

2.4.3 Hazard Index (HI) HI = THQ Ni + THQ_{cd}.........iii

2.4.4 Target Carcer Risk (TRc) TR_c

$$= \frac{EF\ X\ ED\ X\ MI_s\ X\ CPS_0\ \ X\ \ MC}{BW_a\ X\ AT_c} \text{ ....iv}$$

Table 1: Summary Statistic of Input Parameters in the Human Health Risk Evaluation.

Symbol	Description	Unit	Value
MC	Metal Concentration	Mg/kg	Presented in table 2
MI_s	Mass of the snail ingested	Kg/day	0.025
EF	Exposure frequency	Days/year	365
ED	Exposure duration	Years	51.86
RfD	Oral reference dose	Mg/kg/day	Cd=0.003, Ni=0.02
BW_a	Body weight adult	Kg	60

AT_n	Averaging time for non-carcinogens	Days	365x51.86 = 18928.9
AT_c	Averaging time for carcinogens	Days	365x70=25550
PS_o	Carcinogenic potency slop oral	Mg/kg/day	Cd=0.38; Ni=1.7 (subsulfide).

2.4.5 Description / Definition of Terms

CDI: This is the maximum amount of a contaminant to which a person can be exposed to per day over a life time without, an unacceptable risk of health effects.

THQ: Target Hazard Quotient is a ratio of determined dose of a pollutant or contaminant to a reference dose level, and it expressed the risk of non-carcinogenic effects.

HI: Hazard Index is treated as the total sum of the individual metal derived from THQ values of the contaminants. The interpretation of HI values is binary; HI is either $\geq$ I or < I. when HI is > 1, it indicates reason for concerns (that is the exposure population is at risk) while, HI < I signified that the exposure population is at safe limits.[15]

TR_c: Target Carcer Risk indicates carcinogenic risks. The acceptable safe limits of TR according to USEPA and WHO.[14,15,16,17] is $1x10^{-6}$ (one cancer in 1,000,000 people) and 1.0×10^{-4} (one cancer in 10,000 people in some cases).

EF/ED: Exposure frequency/duration this is the number of days per year an individual is exposed to a contaminant over life time which is equal to 365 days/year for 51.86 years (an average life time expectancy of a Nigerian.[3]

$^{AT}{}_n/AT_c$: The averaging time of 365 days/year of 51.86 years for non-carcinogens, and 365 days/year of 70 years for carcinogens. This is an incremental probability of an individual developing cancer over a life time of 70 years.[14,15,16]

RfD is an oral reference dose for non-carcinogens, and C**PSo** the carcinogenic potency slope, oral (mg/kg-bw/day) for Ni and Cd which includes; 0.02, 0.003 and 1.7 and 0.38 respectively [16]. These values describe the upper limits toxicity factors for Ni and Cd for non-carcinogenic and carcinogenic effects.

MI_s/BW_a: is the mass of the snails ingested per body weight per day. The per capita consumption of fish and shell fish in Nigeria for meat and fish products is averaged 9.0k [18] which is equivalent to 0.025 kg per day, 60kg body weight of an adult Nigerian was used in this study.

3.0 RESULTS AND DISCUSSION

3.1 Determination of Nickel and Cadmium in Land and Marine Snails

The mean concentrations and speciation of Ni and Cd in land snails (*A. achatina* and *L. flammea*) and marine snails (*P. aurita* and *T. fuscatus*) of the water soluble fraction (WF_i), non-polar fraction (NF_2), polar fraction (PF_3) and residual fraction (Rf_4) in comparison to standard limits of WHO, FEPA and IAEA [19, 20, 21] are presented in table 2, figure 3 and 4.

Table 2: Mean Concentrations (mg/kg dry wt) of Ni and Cd in the WF_1, NF_2, PF_3, and RF_4 of Land and Marine Snails in Comparison to Standard Limits of WHO, FEPA and IAEA.

Metals fractions (mg/kg)	Snails (mean + StD)			
	A. achatina	***L. flammea***	***P. aurita***	***T. fuscatus***
WF_{NI}	2.00 ± 0.35	1.35 ± 0.44	1.70 ± 1.03	1.55 ± 0.51
WF_{cd}	2.75 ± 2.33	1.30 ± 0.26	0.22 ± 0.02	0.50 ± 0.04
NF_{Ni}	6.50 ± 0.45	2.53 ± 0.02	7.07 ± 0.09	4.51 ± 0.01
NF_{cd}	8.25 ± 0.89	2.50 ± 0.35	0.95 ± 0.39	1.20 ± 0.07
PF_{Ni}	4.27 ± 0.02	1.75 ± 0.07	5.12 ± 0.15	3. 02 ± 0.02
PF_{cd}	6.70 ± 4.40	1.50 ± 0.15	0.57 ± 0.05	0.82 ± 0.40
RF_{NI}	3.32 ± 0.17	1.50 ± 0.07	3.10 ± 0.11	2.13 ± 0.06
RF_{cd}	0.42 ± 0.30	1.20 ± 0.43	0.38 ± 0.06	0.30 ± 0.02
Standard limits	**Ni**	**Cd**		
WHO	0.60	2.00		
FEPA	0.50	2.00		
IAEA	0.60	0.18		

WF_{NI} = Water Soluble Fraction of Ni
WF_{Cd} = Water Soluble Fraction of Cd
NF_{Ni} = Non-Polar Fraction of Ni
NF_{Cd} = Non-Polar Fraction of Cd
PF_{Ni} = Non-polar Fraction of Ni
PF_{cd} = Polar Fraction of Cd
RF_{Ni} = Residual Fraction of Ni, and
FR_{cd} = Residual Fraction of Cd respectively.
WHO = World Health Organization
FEPA = Federal Environmental Protection Agency
IAEA = International Atomic Energy Agency

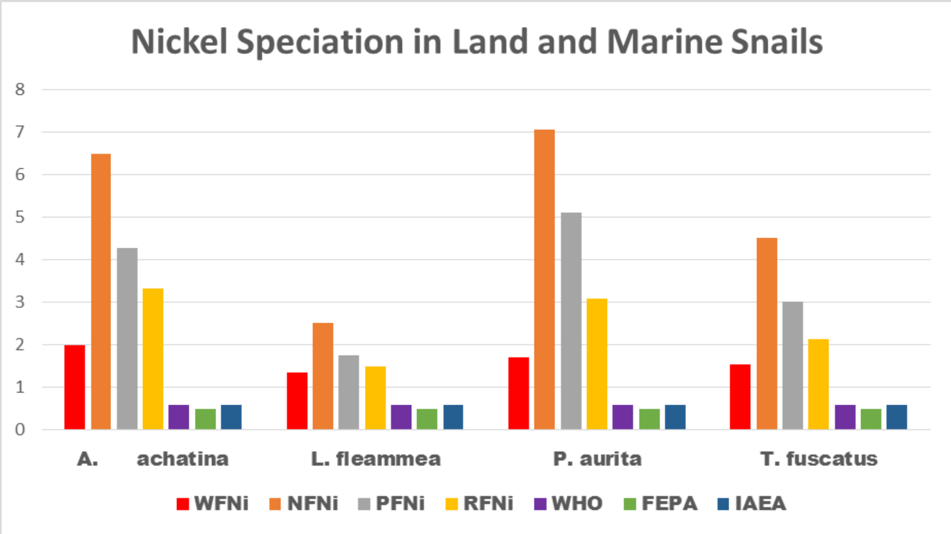

Fig. 3: Speciation of Nickel in the Snail samples (*A. achatina, L. flammea, P. aurita* and *T. fuscatus*) of the different fractions in comparison to standard limits of WHO, FEPA and IAEA.

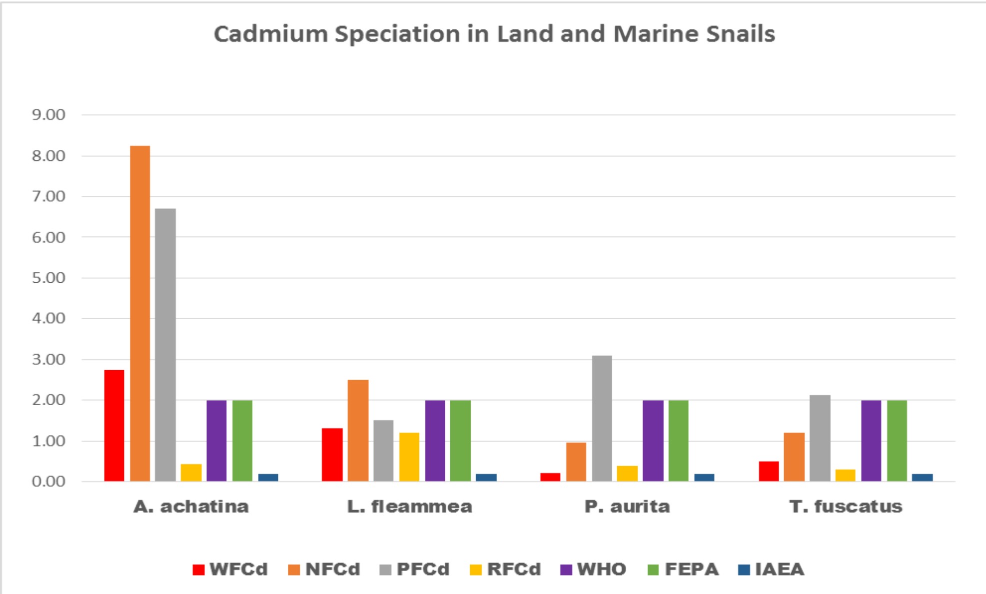

Fig. 4: Speciation of Cadmium in the Snail samples (*A. achatina, L. flammea, P. aurita* and *T. fuscatus*) of the different fractions in comparison to standard limits of WHO, FEPA and IAEA.

The results of the concentrations of Nickel in the different fractions of the snails showed that; WF_{NI} are in the order *A. achatina* > *P. aurita* > *T. fuscatus* > *L. Flammea*. *A. achatina* recorded the highest value of 2.00 mg/kg and *L. flammea* with lowest value of 1.33 mg/kg. NF_{Ni} are in the order of *P. aurita* > *A. achatina* > *T. fuscatus* > *L. flammea*. The highest value of 7.07 mg/kg was found in *P.aurita* and the lowest value of 2.53 mg/kg was found in *L. flammea*. PF_{Ni} also followed the order of *P.aurita* > *A. achatina* > *T. fuscatus* > *L. flammea* with *P. aurita* recording the highest value of 5.12 mg/kg and *L. flammea* recording the lowest value of 1.75 mg/kg. While the RF_{Ni} values followed the order *A. achatina* > *P. aurita* > *T.fuscatus* > *L. flammea*. The highest value of 3.32mg/kg was found in *A. achatina* and the lowest mean value of 2.13 mg/kg was found in *L. flammea.*

Generally, it was observed that the concentration of nickel in the four snails samples were higher in the non-polar fraction, followed by the polar fraction and residual fraction compared to water soluble fraction. This implies that, there are more organic or organickel species in the snails followed by the inorganic species. The comparable amount of nickel found in the residual fraction indicates that some of the nickel complexes (species) are tightly bound to the snail's tissues, and only strong acid digestion can release them.

Nickel can exist in the oxidation states ranging from -1 to +4, but its aqueous chemistry is dominated by the +2 (nickelous) states. These ions form stable complexes with both organic and inorganic ligands and can be absorbed into particular matter. The commonest inorganic liquid ligands are halides, hydroxides, sulphates, phosphates carbonates, nitrate while, the organic ligands are those containing oxygen or sulphur in their structure.[22] The concentrations of Ni in this study in the non-polar, polar and residual fractions are higher than the values reported by Olowoyo.[23] in periwinkles and tilapia in the coastal water of Warri. Also, it is higher than the range value of 0.108 mg/kg in catfish and 0.074 mg/kg in tilapia reported by Ibe *et al.*,[24] from Imo River. Nickel values in the non-polar and polar fractions were also higher than the standard limits of WHO, FEPA and IAEA respectively. The high values of Ni in these snails may pose health hazard (acute and chronic effects) to consumers if consumed continuously.

As indicated in table 2 and figure 2, the mean concentrations of Cd in the different fractions of the snails show that, *A. achatina* has the highest value 2.75 mg/kg and *P. aurita* has the lowest value of 0.22 mg/kg in the WF_{Cd}. Thus the results of the WF_{Cd} values followed the order of *A. achatina* > *L. flammea* > *T. fuscatus* > *P. aurita*. The NF_{Cd} values are in decreasing order *A. achatina* > *L. flammea* > *T. fuscatus* > *P. aurita*. The highest value of 8.25 mg/kg was found in *A. achatina* and lowest value of 0.95 mg/kg was found in *P. aurita*. The highest value of 6.70 mg/kg was also recorded in *A. achatina* and the lowest value of 0.57 mg/kg was recorded in *P. aurita* in the PF_{Cd}. The results of the polar fraction are in the decreasing order of *A. achatina* > *L. flammea* > *T. fuscatus* > *P. aurita.* While in the residuals fraction, the results are in the order of *L. flammea* > *A. achatina* > *P. aurita* > *T. fuscatus*, *L. flammea* ranked the highest with mean value of 1.20 mg/kg, and *T. fuscatus* ranked the lowest with a mean value of 0.30 mg/kg.

Generally, the experimental results from this study shows that the concentrations of Cd in *A. achatina* in all the fractions is higher than other snail samples except in the residual fraction that *L. flammea* ranked the highest, and these high values are found in the non-polar and polar fractions. This implies that, there are more non-polar species of Cd in *A. achatina* followed by the polar species, which also indicates more inorganic and organo cadmium species of these snails [8]. The mean values of *A. achatina* in the polar and non-polar fractions are higher than the standard limits of WHO, FEPA and IAEA respectively. This calls for concern because studies had shown that ingestion of high levels of Cd can lead to acute renal failure in human due to build-up of cadmium in the kidneys overtime, and can also damage the liver, lungs, bone, immune system, blood and nervous system.[25,26]

3.2 Human Health Risk Evaluation of Ni and Cd in the Snails

Food products containing toxic metals could pose a toxic hazard for consumers, and it depends on the metal concentration in the food and the amount of the food consumed daily over a lifetime.[16] In this study, the chronic daily intake (CDI), target hazard quotient (THQ), hazard index (HI) and target carcer risk (TR) risk assessment methods proposed by the United State Environmental Protection Agency (USEPA).[14,15,16] were employed to evaluate the potential human health risks posed by Nickel and cadmium in land and marine snails obtained from Bayelsa State, Nigeria. Thus, the results are calculated and presented in table 3, 4 and 5 respectively.

Table 3: Chronic Daily Intake, CDI (mg/kg-bw/day) of Ni and Cd in Land and Marine Snails in the Different Fractions in Comparison with Standard Guideline Values.

Snail fractions	CDI (mg)kg-bw/day		
	Snail	**Ni**	**Cd**
Water soluble fraction WFI	*A. achantina*	$8.33E^{-04}$	$1.15E^{-03}$
	L. flammea	$5.63E^{-04}$	$5.42E^{-04}$
	P.aurita	$7.08E^{-04}$	$9.17E^{-05}$
	T. fuscatus	$6.46E^{-04}$	$2.80E^{-04}$
Non-polar fraction (NF_2)	*A. achantina*	$2.72E^{-03}$	$3.44E^{-03}$
	L. flammea	$1.05E^{-03}$	$1.04E^{-03}$
	P.aurita	$2.95E^{-03}$	$3.96E^{-04}$
	T. fuscatus	$1.88E^{-03}$	$5.00E^{-04}$
Polar fraction (PF_3)	*A. achantina*	$1.78E^{-03}$	$2.79E^{-03}$
	L. flammea	$7.29E^{-04}$	$6.25E^{-04}$
	P.aurita	$2.13E^{-03}$	$2.38E^{-04}$
	T. fuscatus	$1.26E^{-03}$	$3.42E^{-04}$
Residual fraction	*A. achantina*	$1.38E^{-03}$	$1.75E^{-04}$
	L. flammea	$6.25E^{-04}$	$5.00E^{-04}$
	P.aurita	$1.29E^{-03}$	$1.58E^{-04}$
	T. fuscatus	8.88^{-04}	$1.25E^{-04}$
Guideline standard values FAO/WHO[19]		**0.002-0.04**	**0.003-0.005**

Table 4: Target Hazard Quotient (THQ), Hazard Index (HI) and Percentage Hazard Index (%HI) of Ni and Cd in Land and Marine Snails in Comparison to Standard Values.

Fraction	Snails	THQ		HI	% Contribzution Of Each Metal To HI	
		NI	Cd	Σ THQ	NI	Cd
Water soluble fraction (WFI_1)	*A. achantina*	$3.09E^{-05}$	$2.83E^{-04}$	$3.14E^{-04}$	9.84	90.16
	L. flammea	$2.08E^{-05}$	$1.34E^{-04}$	$1.55E^{-04}$	13.48	86.52
	P.aurita	$2.62E^{-05}$	$2.26E^{-04}$	$4.89E^{-05}$	53.68	46.52
	T. fuscatus	$2.39E^{-05}$	$5.14E^{05}$	7.54^{-05}	31.74	68.26
Non-polar fraction (NF_2)	*A. achantina*	$1.01E^{-04}$	$8.49E^{-04}$	$9.50E^{-04}$	10.60	89.40
	L. flammea	$3.90E^{-05}$	$2.57E^{-04}$	$2.96E^{-04}$	13.18	86.82
	P.aurita	$1.09E^{-04}$	$9.78E^{-05}$	$2.07E^{-04}$	52.75	47.25
	T. fuscatus	$6.96E^{-05}$	$1.23E^{-05}$	$1.93E^{-04}$	36.05	63.95
Polar fraction (PF_3)	*A. achantina*	$6.59E^{-05}$	$6.89E^{-04}$	$7.505E^{-04}$	8.73	11.27
	L. flammea	$2.70E^{-05}$	$1.54E^{-04}$	$1.81E^{-04}$	14.89	85.11
	P.aurita	$7.90E^{-05}$	$5.86E^{-05}$	$1.38E^{-04}$	57.40	42.60
	T. fuscatus	$4.66E^{-05}$	$8.44E^{-05}$	$1.31E^{-04}$	35.59	64.41
Residual fraction (RF_4)	*A. achantina*	$5.12E^{-05}$	$4.32E^{-05}$	$9.45E^{-05}$	54.25	45.75
	L. flammea	$5.12E^{-05}$	$1.23E^{-04}$	$1.47E^{-04}$	15.79	84.21
	P.aurita	$4.78E^{-05}$	$3.91E^{-05}$	$8.69E^{-05}$	55.03	44.97
	T. fuscatus	$3.29E^{-05}$	$3.09E^{-05}$	$6.37E^{-05}$	51.57	48.43
Guideline standard values						
USEPA[16]				**≤ 1**		
FAO/WHO[31]				**≤ 1**		

Table 5: Target Cancer Risk (TR_C) of Ni and Cd in Land and Marine Snail in Comparison to Standard Values.

Fractions	Snails	TR_C		
		Ni	Cd	USEPA and WHO Standard Values
Water soluble fraction (WFI_1)	*A. achantina*	$1.42E^{-06}$	$4.35E^{-07}$	$1.00E^{-04} - 1.00E^{-06}$
	L. flammea	$9.56E^{-07}$	$2.06E^{-07}$	
	P.aurita	$1.20E^{-06}$	3.48^{-08}	
	T. fuscatus	$1.01E^{-06}$	$7.92E^{-08}$	
Non-polar fraction (NF_2)	*A. achantina*	$4.62E^{-06}$	$1.31E^{-06}$	
	L. flammea	$1.79E^{-06}$	$3.96E^{-07}$	
	P.aurita	$5.10E^{-06}$	$1.50E^{-07}$	
	T. fuscatus	$3.19E^{-06}$	$1.90E^{-07}$	
Polar fraction (PF_3)	*A. achantina*	$3.02E^{-06}$	$1.06E^{-06}$	
	L. flammea	$1.24E^{-06}$	$2.38E^{-07}$	
	P.aurita	$3.63E^{-06}$	$9.03E^{-08}$	
	T. fuscatus	$2.14E^{-07}$	$1.30E^{-07}$	
Residual fraction (RF_4)	*A. achantina*	$2.35E^{-06}$	$6.65E^{-08}$	
	L. flammea	$1.06E^{-06}$	$1.90E^{-07}$	
	P.aurita	$2.14E^{-06}$	$1.30E^{-07}$	
	T. fuscatus	$1.51E^{-06}$	$4.75E^{-08}$	

3.2.1 Chronic Daily Intake (CDI)

The chronic daily intake of Ni and Cd via snail's consumption by the average adult of Bayelsa State is presented in table 3. The results showed that, the chronic daily intake of Ni and Cd in all the snails in each fraction, were below the provisional daily intake set by FAO/WHO and USEPA which ranges from 0.002-0.04 mg/kg-bw/day for Ni, and 0.003-0.005 mg/kg-bw/day for Cd,[19,27,28] According to New York State Department of Health (NYSDOH),[29] the risk of a contaminant becomes minimal if the ratio of CDI to its RfD is equal or less than RfD values of the contaminant. Also Singh *et al*, [30] reported that the degree of toxicity of heavy metals to human depends upon the daily intake. The snail species of this present study were found safe for daily human consumption at the moment, however, continuous and excessive daily intake above 0.025mg/kg-bw/day may pose health risks in future.

3.2.2 Target Hazard Quotient (THQ) and Hazard Index (HI).

The target hazard quotient (THQ) and hazard indices for the individual and combined metals through the consumption of the snails in each fraction are presented in table 4. The result shows that, the THQ values of Ni and Cd in the snail species were all below unity (THQ<1). The combined effects of Ni and Cd in the snail species in each fraction were also below the acceptable limits of 1 set by FAO/WHO and USEPA [31, 16]. This indicates no potential non-carcinogenic health risk from a daily ingestion of 0.025 mg/kg-bw/day of Ni and Cd through the consumption of these snail species at the moment. The results of the percentage (%) contribution of each metal to HI in this study shows that Cd was a major risk contributor to the general population exposed to these snails accounting up to 44 - 90% of the total THQ. Therefore, considering the facts that humans are exposed to more than one or two pollutants or contaminants in a given matrix, and often suffer combined or interactive effects,[32] these snails should be consumed moderately to avoid non-carcinogenic effects in future. However, THQ and HI do not directly measure risk because they do not define any dose-response relationship. (USEPA, 1989).

3.2.3 Target Cancer Risk (TRc)

Table 5 shows the results of the target cancer risk of Ni and Cd in the snail species in the various fractions. This study found out that the TRc values of Ni and Cd in all the snail species in each fraction were below the acceptable limits of 10^{-4} and 10^{-6} set by WHO and USEPA.[31,33] This implies that, the human exposure to Ni and Cd via the consumption of these snail species will pose no carcinogenic risk to consumers at the moment. The findings of this study were in agreement with the findings of Markmanuel *et al*,[4] but lower than values reported by Kumar and Mukherjee,[2] in some fish species.

3.2.4 Nickel and Its Compounds

Nickel occurs naturally in the environment at low levels, small amount are found in food, water and air.[34] Nickel is an essential element in some animal species and human nutrition. The major source of nickel exposure, has been reported through food, with average intake of approximately 100-300 micro gram per day (μg/d).[34,35] However, individual may also be exposed to Ni and its compounds via occupation, and everyday items such as jewelry, stainless, steel cooking and eating utensils, and smoking tobacco.[35] Acute effects include lungs and kidney damage which is caused by extreme exposure to high level of Ni via inhalation. Others include gastrointestinal distress (nausea, vomiting, diarrhea), neurological effects (nickel sulphate and nickel chloride) and pulmonary fibrosis and renal edema (nickel carbonyl) [34, 35]. Dermatitis is the most common effect in human from chronic (long-term) dermal exposure to nickel, symptom ranges from the itching of the fingers, hands, wrists, and forearms.[35,36] Also chronic exposure to nickel via inhalation has been reported resulting to respiratory effects such as asthma, decreased lung function, and bronchitis.[35] Animal and human studies have also reported an increased risk of lung and nasal cancer from exposure to refinery dust (nickel oxide), and nickel subslfide.[35] Animal studies of nickel carbonyl (soluble nickel compounds) have reported to cause lung tumor.[36] USEPA has classified nickel refinery dust and nickel subsulfide as Group A, (human carcinogens) and nickel carbonyl as a group B2 (probable human carcinogens) respectively.[37]

3.2.5 Cadmium and Its Compounds.

Cadmium sources in the atmosphere includes burning of fossil fuels such as coal or oil, and incineration of municipal waste materials, and this constitutes the largest sources of cadmium in the environment. Others include air from zinc, lead or copper smelters.[25]

Generally, food has been reported as the largest source of cadmium exposure for non-smokers, and this has been enhanced by the application of phosphate fertilizers or sewage sludge from farm products.[26] Effects on the lungs such as bronchial and pulmonary irritation have been identified as an acute (short term) effects in humans through inhalation, with a single acute exposure to high levels of cadmium resulting in long lasting impairment of lungs function.[25,26,38] While chronic effects (non-cancer) cause kidney diseases such as proteinuria (a decrease in glomerular filtration rate) and an increased frequency of kidney stone formation [25]. These kidney diseases are caused by chronic inhalation and oral exposure of humans to cadmium which results in a build-up of cadmium in the kidney over time.[26] Other effects include, lung, liver, bone, immune system, blood and nervous system damage.[25,26] Animal studies have demonstrated an increase in lungs cancer from long-term inhalation exposure to cadmium but, an increased risk of lungs cancer has not been reported in human studies. However USEPA has classified cadmium as a Group B1, probable human carcinogen.[25,28] Although these snails are safe for human consumption from this present study, however considering the bio-accumulative nature of Ni and Cd in the tissues of organisms, and the potential health risks associated with long-term exposure, these snail species should be consumed moderately.

CONCLUSION

This study was developed in order to provide information on the various forms or species of nickel and cadmium in land and marine snails from Bayelsa State, Nigeria and health risks associated through the consumption of these snails by humans. The experimental results showed that, the concentrations of polar and non-polar fractions of Ni and Cd in the snails ranked the highest with *A. achatina* recording the highest values except in residual fraction of Cd that *L. flammea* ranked the highest. The results of the human health risks evaluation pose no carcinogenic and non-carcinogenic risks at the moment, indicating that, the exposure to Ni and Cd via the consumption of these snails will pose no health risk. However, considering the combine/interactive effects of other pollutants or toxicants in these snails moderate intake is recommended.

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